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14. ABSTRACT

Amplification of 8p11-12 occurs in approximately 15% of human breast cancer (HBC), and this region of amplification is significantly associated with disease-specific survival and distant recurrence in breast cancer patients. Earlier, we used genomic analysis of copy number and gene expression to perform a detailed analysis of the 8p11-12 amplicon for identifying candidate oncogenes in breast cancer. We identified Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1) as a candidate oncogene based on statistical analysis of copy number increase and over expression. In this study, we demonstrated that knockdown of this gene in WHSC1L1 amplified breast cancer cells resulted in profound loss of growth and survival potential. WHSC1L1 contains a PWWP-domain that is a methyl-lysine recognition motif involved in histone code modification and epigenetic regulation of gene expression. To identify genes that may be altered in their expression by over expression of WHSC1L1, we performed expression profiling and identified genes including UHRF1 with altered expression upon WHSC1L1 knockdown in breast cancer cells. We will continue to investigate how WHSC1L1 contributes to transformation through the alternation of epigenetic histone marks and acquisition of stem cell-like properties in breast cancer cells.

15. SUBJECT TERMS

Gene amplification, PWWP-domain, histone modification

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Introduction

Amplification of 8p11-12 occurs in approximately 15% of human breast cancer (HBC), and this region of amplification is significantly associated with disease-specific survival and distant recurrence in breast cancer patients (1-5). Earlier, we used genomic analysis of copy number and gene expression to perform a detailed analysis of the 8p11-12 amplicon for identifying candidate oncogenes in breast cancer (4). We identified Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1) as a candidate oncogene based on statistical analysis of copy number increase and over expression (4). The WHSC1L1 gene encodes a PWWP domain protein that regulates gene transcription and differentiated function of cells through regulation of histone methylation (6, 7). In this proposal, we hypothesize that WHSC1L1 is the major driving oncogene in the 8p11 amplicon that is found in aggressive forms of ER positive, luminal breast cancers. Further, we hypothesize that genetic deregulation of WHSC1L1 induces alterations in the epigenetic histone code resulting in the acquisition of cancer stem cell phenotypes based on the transcriptional changes that result from altering histone methylation patterns in breast cancer cells. Based on this hypothesis, we predict that WHSC1L1 will be a good therapeutic target in breast cancer, particularly for those ER positive breast cancers that are, or become refractory to endocrine therapy.

Body

1. Specific Aims

This project consists of 3 specific aims:
Aim 1: To investigate the molecular mechanism, including the structural details, of WHSC1L1 that are involved in transforming function through the alteration of the epigenetic histone code in human breast cancer cells.
Aim 2: To determine whether the histone modulation function of WHSC1L1 is linked to cancer stem cell phenotypes.
Aim 3: To examine the potential of WHSC1L1 as a therapeutic target in aggressive, ER-positive breast cancers that harbor the 8p11 amplicon.

2. Studies and Results

***Task 1*. To investigate the molecular mechanism, including the structural details, of WHSC1L1 that are involved in transforming function through the alteration of the epigenetic histone code in human breast cancer cells**. *Month 1-16*

Expression of the WHSC1L1 gene results in two alternatively spliced variants, a long isoform and a short isoform that are derived from alternative splicing of exon 10 . The WHSC1L1 long isoform encodes a 1437 amino acid protein containing 2 PWWP domains, 2 PHD-type zinc finger motifs, a TANG2 domain, an AWS domain and a SET domain. The short isoform encodes a 645 amino acid protein containing a PWWP domain only. In our previous annual report, we demonstrated that stable overexpression of WHSC1L1 short isoform in the non-tumorigenic breast cell line MCF10A induces a transformed phenotype whereas knock-down in tumor cells inhibits proliferation, supporting WHSC1L1 as a transforming oncogene in 8p11-12 amplified breast cancer. To elucidate the relationship between the transforming function and specific structural motifs, particularly the PWWP binding and SET enzymatic domains, we established a series of WHSC1L1 constructs with or without SET domains. Each of these constructs had been incorporated into the lentiviral expression system. MCF10A cells stably over expressing different truncated forms of WHSC1L1 have been established and protein expression confirmed by Western blot (Figure 1). Our preliminary data revealed that over expressing the long-isoform of WHSC1L1 in

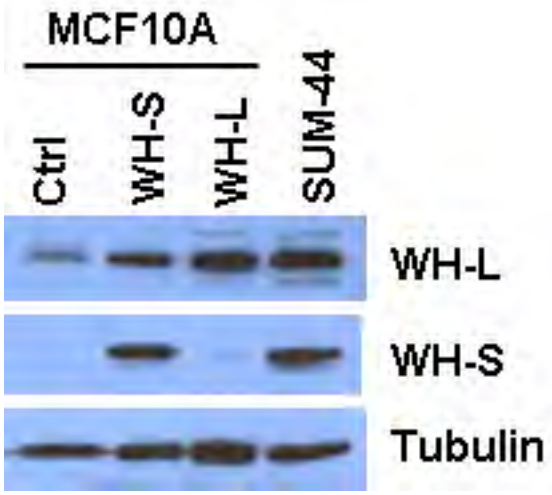

Figure 1. Expression of WHSC1L1 long-isoform (WH-L) and short-isoform (WH-S) in MCF10A cells that were infected by different forms of WHSC1L1, as well as WHSC1L1 amplified SUM-44 cells was analyzed by the Western blot.

MCF10A cells also induces growth factor independent proliferation, similar to over expressing the short isoform of WHSC1L1 in MCF10A cells. Next, these model cells will also be used to determine the genome-wide distribution of the histone modifying protein WHSC1L1 in mammary epithelial cells by ChIP-on-chip assays.

Task2. **To determine whether the histone modulation function of WHSC1L1 is linked to cancer stem cell phenotypes**. Months 12-30

The cancer stem cell (CSC) hypothesis suggests that a subset of tumor cells with stem-cell-like properties is primarily responsible for the growth, progression and recurrence of cancer (8-10). Alterations in histone methylation and demethylation are likely to be critical steps in neoplastic progression by disrupting the normal stem/progenitor cell program. Use of specific cell-surface markers allows for the identification and enrichment of normal stem cells and tumor-initiating cells from tissues and cell lines. Several groups identified a subpopulation of cells in human breast cancer with the phenotype CD24-/low/CD44+ that display stem cell properties. More recently, measuring the expression of aldehyde dehydrogenase (ALDH), an enzyme previously found to be expressed in hematopoietic and neuronal stem cells, has been established as a new tool to detect normal and malignant human mammary stem cells (11, 12). ALDH can be assessed by the Aldefluor assay to detect cells displaying aldehyde dehydrogenase activity (Stem Cell Technologies, Inc). We have tested several specific stem cell surface markers including aldehyde dehydrogenase (ALDH) by using an immunohistochemistry staining assay in breast cancer cells (Figure 2). Next, we will use these methods to determine if altered expression of histone-modifying protein WHSC1L1 results in expansion/contraction of cancer stem cell pools.

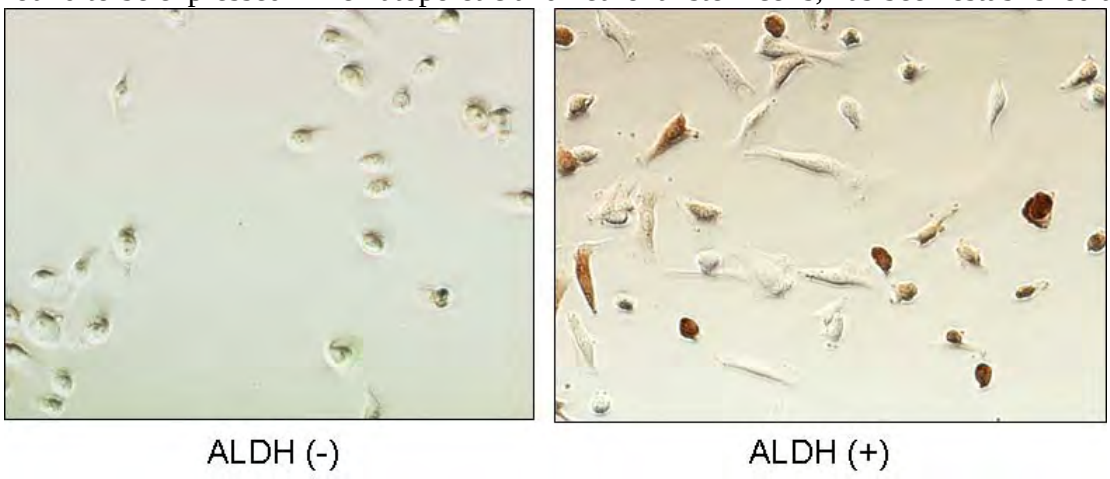

Figure 2. Expression of the cancer stem cell marker ALDH was detected by immunohistochemistry staining in two breast cancer cell lines.

Task 3. **To examine the potential of WHSC1L1 as a therapeutic target in aggressive, ER-positive breast cancers that harbor the 8p11 amplicon.** Months 18-36

In our previous report, we examined the effects of knock down of WHSC1L1 with shRNAs in SUM-44 and SUM-52 cells where WHSC1L1 is amplified and over

expressed, and in the control cell line MCF10A. We domonstrated that WHSC1L1 knock-down suppressed proliferation of SUM-44 and SUM-52 cells, while WHSC1L1 shRNAs had no effect on the growth of MCF10A cells. In the current period, we performed expression profiling of SUM-44 cells with or without WHSC1L1 knockdown. To perform RNAi knock-down experiments, we identified the two most efficient shRNAs with respect to knock-down of WHSC1L1 expression levels in SUM-44 cells. Q-RT-PCR and western blot data revealed that the WHSC1L1-shRNAs #2 and #6 resulted in decreases in mRNA and protein levels to approximately 20-30% of the level seen in the non-silencing control-infected cells (Figure 3). Next, to identify genes with altered expression upon WHSC1L1 knockdown, we performed genome-wide expression profiling analysis. Knockdown of WHSC1L1 in SUM-44 cells yielded 80 down-regulated genes and 66 up-regulated genes with at least a two-fold change relative to control (Table 1). A finding of particular interest from our current study is that UHRF1 (ubiquitin-like with PHD and ring finger domains 1) is a candidate target of WHSC1L1. Recent studies demonstrated that UHRF1 has the ability to bind hemi-methylated DNA and methylated H3K9 through its SRA domain and Tudor domain, respectively (13-15). UHRF1 can repress transcription of tumor suppressor genes including *p16*^{INK4a} and *p21*$^{Waf1/Cip1}$ via recruitment of DNA methyltransferases (DNMT1 and DNMT3A/B), H3K9

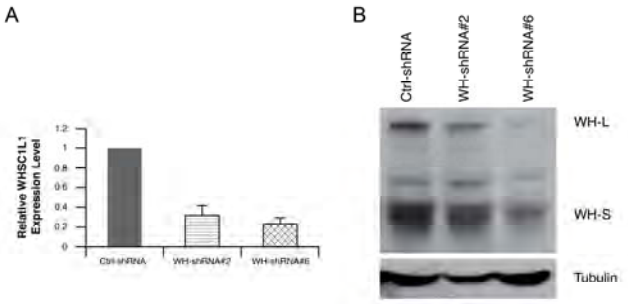

Figure 3. WHSC1L1 expression in SUM-44 cells was analyzed by (A) semiquantitative RT-PCR and (B) western blot after infection with non-silencing control shRNA or WHSC1L1 specific shRNA (shRNA#2 and #6).

Table 1. The down- and up-regulated genes in SUM-44 cells after knocked down WHSC1L1 with specific shRNA (shRNA#2 and #6)

Down-regulated			Up-regulated		
Gene	WHSC1L1-sh#2	WHSC1L1-sh#6	Gene	WHSC1L1-sh#2	WHSC1L1-sh#6
MCM10	-3.39	-1.15	HIST1H2AC	2.26	1.22
UHRF1	-3.33	-1.15	P8	2.23	1.11
SCGB1D2	-3.32	-2.31	HMOX1	2.22	1.36
CDC45L	-3.16	-1.27	HIST1H2BD	2.18	1.08
CXCL10	-3.10	-2.46	PLAC1	2.15	1.08
DTL	-2.83	-1.26	HIST1H2BD	2.05	1.38
MCM2	-2.82	-1.12	TIGA1	2.03	1.29
MCM6	-2.76	-1.19	RPS29	2.00	1.77
EXO1	-2.71	-1.20	IFI27	1.96	1.91
E2F2	-2.61	-1.04	HS.571151	1.92	1.43
FEN1	-2.51	-1.10	CREB5	1.71	1.21
IL8	-2.51	-1.56	HIST1H4H	1.68	1.12
GINS2	-2.41	-1.08	NPC1L1	1.66	2.15
CCNE2	-2.40	-1.19	KRT80	1.60	2.24
CDT1	-2.38	-1.25	HS.408455	1.58	1.28
IL8	-2.34	-1.83	MGC4677	1.58	1.09
SPINK4	-2.26	-2.15	HS.576428	1.56	1.77
AKR1C2	-2.07	-2.37	STOM	1.51	1.12
CCL20	-2.06	-2.33	RGS2	1.50	1.70
MCM3	-2.04	-1.03	DTNA	1.49	2.39
ASF1B	-2.02	-1.12	C15ORF48	1.48	1.24
CES1	-1.98	-2.23	COLEC12	1.42	3.14
EXO1	-1.98	-1.19	HSD17B2	1.41	1.05
BRI3BP	-1.98	-1.11	HIST1H1C	1.41	1.09
SLC27A2	-1.94	-2.26	SHISA2	1.40	2.30
ADORA1	-1.92	-1.39	KIF5C	1.33	1.46
OKL38	-1.91	-2.24	MIPEP	1.32	1.25
IL17RB	-1.88	-1.31	TTC32	1.27	1.02
NDST4	-1.84	-3.15	KLF6	1.26	1.01
S100A8	-1.83	-1.51	STOM	1.25	1.04
TIPIN	-1.82	-1.31	PIK3IP1	1.24	1.53
SLC5A8	-1.80	-2.71	HS.374460	1.24	1.02
MCM4	-1.76	-1.16	RPL13A	1.23	1.02
CDC25A	-1.76	-1.11	MS4A7	1.23	1.67
WHSC1L1	-1.75	-1.12	NTN4	1.22	1.11
PDZK1	-1.74	-2.09	MAP1LC3A	1.22	1.03
RET	-1.72	-1.50	FLOT1	1.22	1.41
FKBP4	-1.72	-1.65	CPVL	1.20	1.23
KNTC1	-1.68	-1.09	C9ORF150	1.20	1.43
S100A9	-1.68	-1.92	ZFP36L1	1.18	1.16
UNG	-1.63	-1.05	HS.544637	1.17	1.01
RGS22	-1.62	-1.59	OSR2	1.15	1.30
POLA2	-1.62	-1.28	CPVL	1.15	1.34
SLC39A6	-1.60	-1.67	DOCK2	1.14	1.14
RFC4	-1.59	-1.07	C1ORF97	1.14	1.18
TNF	-1.58	-1.41	PLCE1	1.13	1.58
CHAF1B	-1.58	-1.05	HIST2H2AC	1.13	1.21
GINS3	-1.55	-1.17	CXCR7	1.13	1.24
BASP1	-1.52	-1.18	HS.13291	1.12	1.24
NQO1	-1.52	-1.28	PIK3R1	1.11	1.14
TREML3	-1.51	-1.43	ABCC5	1.10	1.29
INSM1	-1.48	-1.49	WNT11	1.09	2.16
OSGIN1	-1.47	-1.73	LOC388135	1.08	1.26
NR4A2	-1.47	-2.02	DIO1	1.08	1.36
S100A7	-1.46	-1.22	CENPA	1.07	1.02
C6ORF192	-1.44	-1.11	LOC130576	1.07	1.13
GPNMB	-1.42	-1.39	HRASLS3	1.07	1.01
TFG	-1.42	-1.62	CCNG2	1.07	1.32
C16ORF59	-1.36	-1.11	PTTG3	1.06	1.21
RET	-1.35	-1.43	INSIG2	1.05	1.02
LOC442117	-1.31	-1.15	FMO9P	1.05	1.29
H2AFY	-1.30	-1.11	IRF6	1.05	1.47
NR4A2	-1.28	-1.74	SYTL2	1.04	1.14
GPNMB	-1.27	-1.03	HIST2H2AA3	1.03	1.21
SPRY4	-1.27	-1.25	NRP1	1.02	1.87
ADORA1	-1.27	-1.25	FLRT3	1.02	1.52
IER3	-1.27	-1.40			
ASCL1	-1.20	-1.26			
FAM46A	-1.16	-1.03			
FGFR4	-1.15	-1.47			
SLC39A6	-1.15	-1.30			
SGK3	-1.14	-1.01			
UCHL5IP	-1.14	-1.01			
RFC3	-1.14	-1.05			
ISG20L1	-1.14	-1.02			
HDC	-1.13	-1.19			
PXMP4	-1.08	-1.24			
MAFF	-1.08	-1.04			
ELP2	-1.07	-1.52			
PRSS1	-1.02	-1.26			
ABCC12	-1.01	-1.75			

methyltransferases (G9a), and HDAC1, interconnecting DNA methylation and histone modification pathways (16, 17). Next, we will perform ChIP-PCR and ChIP-on-chip experiments to determine whether WHSC1L1 directly regulates these genes including UHRF1 through its histone modulation activity (**Aim 1 and Aim 3**).

Key Research Accomplishments

In the present study, we systematically investigated the transforming properties of the newly identified 8p11-12 candidate oncogenes WHSC1L1 *in vitro*. Knockdown of *WHSC1L1* in 8p11-12 amplified breast cancer cells resulted in profound loss of growth and survival of these cells. We performed expression profiling and identified genes including UHRF1 with altered expression upon WHSC1L1 knockdown in breast cancer cells. Further, we established methods and models for determining whether the histone modulation function of WHSC1L1 is linked to cancer stem cell phenotypes.

Reportable Outcomes

Manuscript:
Yang ZQ, Liu G, Bollig-Fischer A, Giroux CN, Ethier SP. Transforming properties of 8p11-12 amplified genes in human breast cancer. Cancer Res. 2010;70:8487-97.

Conclusion

We have made significant progress in the past year in characterizing the PWWP-domain protein WHSC1l1 in human breast cancer. We revealed that knockdown of this gene in WHSC1L1 amplified breast cancer cells resulted in profound loss of growth and survival potential in these cells. The PWWP-domain is a methyl-lysine recognition motif involved in histone code modification and epigenetic regulation of gene expression. To identify genes that may be altered in their expression by over expression of the WHSC1L1, we performed performed the expression profiling and identified genes including UHRF1 with altered expression upon WHSC1L1 knockdown in breast cancer cells. In the next year, we will continue to investigate how WHSC1L1 contributes to its transformation through the alternation of epigenetic histone marks and acquisition of stem cell-like properties in breast cancer cells.

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